# FEATURE-AIDED PARTICLE TRACKING

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## ABSTRACT

We present a new feature-aided tracking algorithm dedicated to the task of tracking multiple and closely-spaced biological particles. We propose a new function to score associations, based on kinetic models, and enriched with an additional feature. This feature is based on adaptive profiles and the physical properties of the acquisition system. A key property is that this feature definition allows to resolve the challenging task of tracking particles that appear fused.

Results on simulations show improved performances over existing methods both on tracking and on the resolution of fused particles.

*Index Terms*— particle tracking, feature-aided tracking, optical transfer function, super-resolution

## 1. INTRODUCTION

Live video-microscopy combined with automatic particles tracking is opening new ways to study and understand intracellular processes by taking advantage of recent developments in particle tracking methods [1, 2]. The classical paradigm of particle tracking is a detection step followed by an association procedure between the measurements set and the active tracks set. A scoring function is used to validate association hypotheses coming from different models.

In the **kinetic Bayesian approach** the association score is based on the kinetic likelihood of each association and additional event statistics such as false measurement and new target birth. This is usually achieved thanks to the definition of a *state space* of the targets [3] and a Kalman filtering technique. It has been shown to give good results in many biological applications because of its robustness to noise and its capacity to take into account kinetic models of the targets' motion [4]. In several practical cases however the kinetic likelihoods are very similar for many association hypotheses, especially when the measurements are very close to each other and the targets dynamics non discriminant. In such challenging cases, the performance of Bayesian kinetic tracking quickly degrades.

A different class of tracking methods relies on the use of **template matching**, where the association is performed by maximization of the agreement between the targets templates and the current image. While these methods give good results when targets profiles are discriminant, they suffer from the lack of kinetic models when targets are nearly similar or when the noise level is high.

These two ways of scoring an association hypothesis seem highly complementary, which is why ideas of enriching the kinetic Bayesian

approach with non kinetic data have emerged among the radar tracking community, namely *feature aided tracking* and *classification aided tracking* [5, 6]. They have been shown to outperform pure kinetic or pure template tracking.

For the tracking of biological targets, improvements were achieved by the use of the area and mean intensity in a kinetic Kalman filter (KAI) [1]. We will however show in Section 2 that KAI is not able to handle several biological cases. We therefore present in Section 3 a new Bayesian tracking algorithm enriched with an additional feature. This feature is based on the acquisition system knowledge and adaptive profiles (PA). A key property of this feature definition is that it allows to **resolve the challenging task of tracking particles that appear fused** as described in Section 4.

In Section 5 we validate the capacity of our algorithm to resolve closely spaced targets, and quantitatively compare PA and KAI tracking results on simulated images.

## 2. BAYESIAN FEATURE ADAPTED TRACKING

In a Bayesian tracking algorithm the association score function between the set of measurements at time $k$: $Z(k) = \{z_i(k)\}_{i=1..m_k}$, and the set of active tracks is classically chosen to be the likelihood of the association given all past measurements $P\{\Theta(k)|Z^k\}$, where $Z^k$ is the vector of measurements up to time $k$ and $\Theta(k)$ is an element of the set of feasible associations at time $k$: $\Omega(k)$. The following derivation given in [3] is commonly used:

$$P\{\Theta(k)|Z^k\} = P\{\Theta(k)\}\frac{1}{c}p[Z(k)|\Theta(k), Z^{k-1}] \qquad (1)$$

$$= P\{\Theta(k)\}\frac{1}{c}\prod_{j=1}^{m_k} p[z_j(k)|\theta_{jt_j}(k), Z^{k-1}] \qquad (2)$$

where c is constant across the association hypotheses. $P\{\Theta(k)\}$ is the probability that the hypothesis $\Theta(k)$ is true and depends on data such as the number of associations, false alarms and track births. The first derivation step (1) is the foundation of the iterative scheme and the second step (2) is achieved under the assumption of conditional independence of measurement process for each target. Each likelihood of individual association $p[z_j(k)|\theta_{jt_j}(k), Z^{k-1}]$ between measurement $z_j(k)$ and track $t_j$ is given by the kinetic model of the track.

The idea of feature adapted tracking consists in introducing the feature information in the score function by splitting the measurement vector $Z(k)$ into a kinetic part $Y(k)$ and a features related part $S(k)$: $Z(k) = [Y(k)\ S(k)]$. From Equation (2) and by considering the features as some characteristics of each individual association, the

likelihoods of features and kinetic data can be written as:

$$p[z_j(k)|\theta_{jt_j}(k), Z^{k-1}] = p[s_j(k)|y_j(k), \theta_{jt_j}(k), Y^{k-1}, S^{k-1}]$$
$$\cdot p[y_j(k)|\theta_{jt_j}(k), Y^{k-1}, S^{k-1}] \quad (3)$$

Equation (3) isolates the feature $s(k)$ from the kinetic information $y(k)$, but a strong requirement of this decomposition is the knowledge of the features distribution. This knowledge is critical because the behavior of the score function depends directly on it and the weight of the feature information is linked to its parameters.

In the standard Bayesian tracking of biological particles the first step is objects detection which usually provides few feature measurements such as area and intensity of targets. Both have been used in the association score function by introducing them in the state space model of a standard kinetic Kalman filter [1] using Equation (3). While performance is good some drawbacks exist: A Gaussian distribution has been chosen in [1], but little information supports this assumption. The parameters of this distribution are not known a priori so many trials are necessary to find values that seem appropriate, but with no guarantee on the proper behavior of the score function.

In practice we have also observed behaviors of area and intensity that cannot be taken into account by the state space model, which leads to early tracks termination or erroneous tracks. In the next part we will present a feature choice that avoids these drawbacks and limitations.

### 3. ADAPTIVE PROFILES AIDED TRACKING

#### 3.1. Using profiles information

In optical imaging the Point Spread Function (PSF) of the acquisition system gives the response from a Dirac point source, and its use in building a feature is therefore very powerful: under the assumption of a Dirac target the PSF synthesizes in a single function the usual detection descriptors such as area and intensity. For sub-resolutive targets it has been proved that the PSF of commonly used acquisition systems such as 2d and 3d Laser Scanning or Disk Scanning microscopy, and 2d wide-field microscopy, can be almost perfectly approximated by a Gaussian in 2d and two Gaussians in 3d [7]. This model is very convenient because of its compactness and its analytical expression allowing the use of efficient optimization techniques. Each target can thus be identified by its own set of parameters of a Gaussian profile that depends on its size, the number of photons it emits, its defocalization in 2d microscopy. We could try to use the parameters of the Gaussian profile estimated in an image as a feature in a kinetic-feature mixed Kalman filter, but this would still suffer from the lack of knowledge of the dynamics of profile parameters distribution. We propose a method that exploits these profiles information in what we think is a much more appropriate manner.

#### 3.2. Residual image as a feature in scoring function

In many biological images an additive model of the images is suitable, it describes intensities at time $k$ $\{I_k^{th}(x,y)\}_{x,y}$ as a superposition of targets profiles $\{P_{i,k}\}_i$, the stochastic acquisition noise $N_k(x,y)$ and a constant background value:

$$I_k^{th}(x,y) = N_k(x,y) + b_k + \sum_i P_{i,k}(x,y) \quad (4)$$

The center of each profile's function is given by the tracking association hypothesis at time $k$: $\Theta(k)$. Therefore if this hypothesis is

true, the image model (4) would closely agree with the observed image $I_k^{obs}$, and, on the contrary, if the association is false the observed image would differ from the model. Under the assumption of true association there is no difference between the image model and the observed image (see Eq. 5). Therefore the residual between the observed image, the profiles intensities and the background follows the same distribution as the noise (Eq. 6):

$$I_k^{obs}(x,y) - I_k^{th}(x,y) = 0 \quad (5)$$

$$I_k^{obs}(x,y) - \sum_i P_{i,k}(x,y) - b_k = N_k(x,y) \quad (6)$$

From this, and this is a key point of the method, we can estimate the likelihood of an association hypothesis based on the likelihood of the residual $\{R_k(x,y)\}_{x,y}$ defined as:

$$R_k(x,y) = I_k^{obs}(x,y) - \sum_i P_{i,k}(x,y) - b_k \quad (7)$$

because under the true association hypothesis, residual $\{R_k(x,y)\}_{x,y}$ follows the distribution of the acquisition system noise, which is a well defined property of many optical acquisition systems. Usually it is described as being white, Gaussian, or Poisson, or a mixture of both [8]. Next we will consider the white Gaussian case only, because we pre-process images with a Variance Stabilizing Transform [8] which forces noise to follow a Gaussian distribution.

We now define an alternative to Equation (3) for deriving the likelihood of an association where the feature likelihood is isolated at the global association hypothesis level:

$$P_{PA}\{\Theta(k)|Z^k\} = \frac{1}{c} p[S(k), Y(k)|\Theta(k), Z^{k-1}] P\{\Theta(k)\}$$
$$= \frac{1}{c} p[S(k)|Y(k), \Theta(k), Z^{k-1}]$$
$$\cdot p[Y(k)|\Theta(k), Z^{k-1}] P\{\Theta(k)\} \quad (8)$$

A feature does not characterize here a single association, but the overall associations between tracks and targets. Equation (8) isolates the feature contribution from the kinetic likelihood, and the latter can be decomposed in a product of kinetic likelihood of single association in the very same way as described by the right hand side of Equation (2).

If the acquisition frequency is sufficiently high we can approximate the current profiles parameters by those estimated at previous times: $\{P_{i,k}\}_i \approx \{P_{i,k-1}\}_i$, which makes our approach adaptive to profiles variations. The feature-tracking procedure at time $k$ can then be summarized by the following:

1. Estimate profiles parameters of targets at time $k-1$: $\{P_{i,k-1}\}_i$,

2. Build a set of kinetic feasible associations $\Omega(k)$ between tracks and targets at time $k$ and compute their likelihood $p[Y(k)|\Theta(k), Z^{k-1}]$ for each $\Theta(k) \in \Omega(k)$,

3. For each hypothesis build the residual image at time $k$: $\{R_k(x,y)\}_{x,y}$ based on estimated profiles and hypothesis positions and compute its likelihood $p[S(k)|Y(k), \Theta(k), Z^{k-1}]$,

4. Compute the score of the association based on the kinetic and feature data: $P_{PA}\{\Theta(k)|Z^k\}$.

1797

### 3.3. Residual likelihood computation

In our applications the acquisition noise variance is not known a priori, we thus have to evaluate it from images. We have used the p-value of the hypothesis test concerning the equality of variance of two samples as our likelihood score of the residual $\{R_k(x,y)\}_{x,y}$ because it allows us to take into account the variability of variances estimations. For this, we test the equality of the variance of the residual $\sigma_r^2$ to the variance of the noise $\sigma_n^2$:

$$\left\{ \begin{array}{l} H_0 : \sigma_r^2 = \sigma_n^2 \\ H_1 : \sigma_r^2 > \sigma_n^2 \end{array} \right. , \quad T = \frac{\sigma_r^2}{\sigma_n^2} \propto F \qquad (9)$$

The $T$ statistics is Fisher distributed with degrees of freedom determined by the size of images used to estimate the two variances. If the p-value is high the likelihood of Equality (6) and consequently that of the true association are both high.

The weight of the feature information in $P_{PA}$ depends on the noise level that is used to compute the $T$ statistics, this is an implicit way to take into account the confidence in intensity information.

## 4. NON-RESOLVED TARGETS SEPARATION

When the targets density increases, classical multi-targets tracking algorithms performance dramatically degrades due to two main problems: first the probability of incorrect association increases, and second the detection step results are less accurate. The latter occurs because overlaps of targets profiles are frequent and standard detection procedures based on filtering and thresholding are not able to resolve them. Nevertheless it has been shown that this resolution limit can be broken when PSF information is fully exploited. For instance in [9], with a goodness-of-fit bottom-up approach, the PSF information is used to determine the number of targets in an image, even if they are closely spaced.

The tracking procedure can help solving this detection problem, indeed when at a given time the number of measurements is lower than the number of existing tracks, we could conclude that some targets were not detected in the image, but we also could suspect non-resolved targets overlapping due to their proximity. We therefore propose to introduce some fusion hypotheses in the set of feasible association hypotheses $\Omega(k)$.

Fusion hypotheses are built by adding positions near pre-detected ones given by each $\Theta(k) \in \Omega(k)$, and then by least-square optimization of the set of positions using the set of adaptive target profiles $\{P_{i,k}\}_i$. The number of fused particles in an aggregate is limited by the number of active tracks for which its kinetic filter allows this association. Each association hypothesis, whether it contains a fusion or not, can be scored uniformly by the profile adapted function $P_{PA}$, with incorrect fusion events being naturally penalized by a low likelihood of the residuals $\{R_k(x,y)\}_{x,y}$. The tracking procedure can then proceed with the hypothesis selection step using scores given by $P_{PA}$.

## 5. RESULTS

We present here the validation of the PA score function and of the fusion recovery procedure. It consists on various experiments on synthetic data because these ones have the advantage of being completely controlled which allows an accurate study of the behavior of the method and a systematic comparison to existing solutions.

We nevertheless tried to reproduce with fidelity the conditions of a common biological experiment. In order to simulate a typical acquisition of 2d images with a confocal laser scanning microscope we used a realistic PSF model (given in [7]) for the non paraxial case (numerical aperture of 0.5), a pixel size of 100 nm and an emission wavelength of 388 nm, which are common imaging settings for biological studies. Assuming targets as Dirac sources, then differences of profiles are due to variations of focalization and photo emission between targets. Acquisition time length is 25 seconds, with an acquisition frequency of 4 frames per second.

We use a standard Bayesian tracking procedure using an Interacting Multiple Models (IMM) filter and a minimal set of decision rules for track confirmation and termination in order to compare score functions (PA and KAI) performances and to investigate the ability to recover fusion events.

### 5.1. Fusion event recovery

The capacity of the algorithm to recover fusion events is first evaluated. For this we simulated a simple fusion between two particles by setting the distance between the two targets to 100 nm at a given time. This distance is two times less than the Rayleigh resolution limit defined by the PSF, which makes targets to appear fused to a simple thresholding detection technique. In order to isolate the fusion event from other interactions between the particles we have set their mode of motion as being a superposition of diffusive movements (0.8$\mu$m/s) and perpendicular directed (2$\mu$m/s) movements.

We have generated 200 simulations for various Signal to Noise Ra-
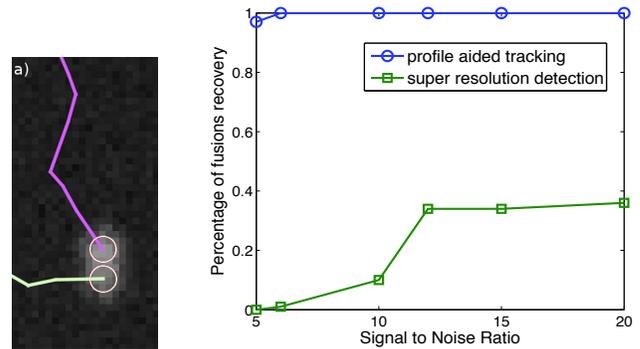


**Fig. 1**. Fusion events recovery. (a) Separation of two closely spaced targets in an image with SNR =20. (b) Fusion recovery performance of proposed tracking algorithm and super resolution algorithm.

tio (SNR) values, the results of the number of fusion recoveries under these conditions are presented in Figure 1. Unfortunately performance under lower SNR is difficult to analyse because results of pre detection become very poor. This graph also shows the number of fusion events recovered by the super-resolutive detection algorithm introduced [9], it is striking to see that whatever the SNR condition we have tested the PA tracking algorithm recovers nearly 100% of the fusion events, while the super-resolution algorithm most of the time detects only one target.

The superior performance of the tracking algorithm shows that when information made available by the tracking procedure, such as kinetic models, is combined with profiles information that is specifically estimated for each target in our method, the resolution limit is

1798

clearly beaten. This is a major benefit of the use of PA score function: it combines in an unified score the overall information available.

## 5.2. High density environment

We have simulated a high density environment by placing 25 targets in a small area, the particles movements are observed for 25 s, they all have a Brownian motion whose diffusion coefficient is obtained by a small random perturbation of the reference diffusion coefficient: 1.6 $\mu$m/s. See Figure 2. Profiles are given by random perturbations of the reference PSF. The task of tracking these particles is especially
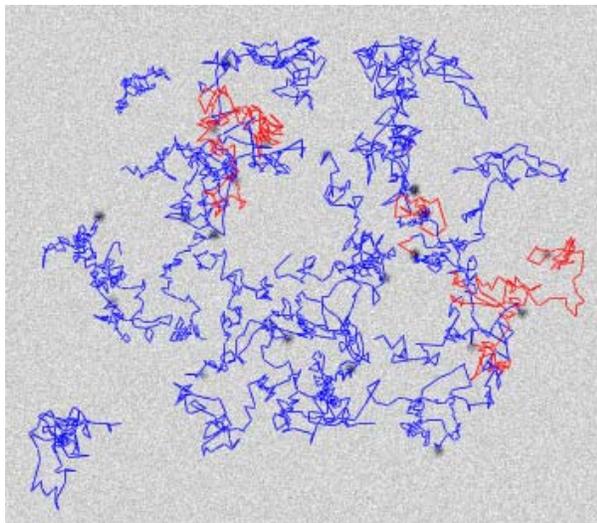


**Fig. 2**. High density environment simulation. Recovered tracks by tracking algorithm using PA score are in blue, lost tracks are in red.

|  | KAI | PA |
|---|---|---|
| number of complete tracks | **109**/250 | **180**/250 |
| mean track length | 71.64/100 | 85.6/100 |

**Table 1**. Tracking results for closely spaced tracks.

difficult, first because the density of targets is high, which makes fusion events and possible false associations numerous, and second because Brownian kinetics are low informative. Tracking results on 100 simulations are presented in Table 1, it shows poor performances of the classical method with the KAI score, while PA score significantly outperformed it, which shows in a realistic environment the benefits of the proposed approach.

## 6. CONCLUSION

We have proposed a new way to combine in a unified framework two classical tracking approaches: kinetic Bayesian tracking and profiles matching tracking. We have been able to take advantages of the classical feature-aided tracking, and to avoid the major drawbacks of the intensity-and-area-aided tracking in biological images. The use of the PA score function in any tracking scheme is made easy because

there is no extra parameter to tune and no special procedure of decision. In particular the weighting of residual information and kinetic model in $P_{PA}$ is implicit.

On top of this the very challenging problem of non resolved targets due to close proximity is also solved thanks to the use of informations given both by profiles and by past events and kinetic models. These additional hypotheses of association are taken into account thanks to our choice of a function that scores in an unified manner 'simple' hypotheses and 'fused' hypotheses with no additional decision procedure.

Since these desirable properties have been confirmed and show a superior performance over KAI score function on synthetic data, we have started an extensive study on real biological data. Extension to 3D will be addressed and studies of mixed score functions behavior will be investigated further.

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